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The effects of substratum on locomotor performance in lacertid lizards

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Locomotion is important to animals because it has direct implications for fitness through its role in predator escape, prey capture, and territory defence. Despite significant advances in our understanding of animal locomotion, studies exploring how substrate properties affect locomotor performance remain scant. In the present study, we explore how variation in substrate (sand, slate, cork) affects locomotor performance in lacertid lizards that differ in morphology. Moreover, we explore whether substrate effects are the same for different types of locomotor performance (speed, acceleration, and stamina). Our results show that the substrate affected most types of locomotor performance studied but not always in the same way. Although substrate effects were species-dependent for the maximal speed over 50 cm and the distance run to exhaustion, this was not the case for acceleration capacity. These results suggest that substrate texture differentially affects burst performance vs. longer duration measures of locomotor performance. Finally, straightforward relationships between habitat use and the substrate on which performance was maximized were not observed. This suggests that the evolution of locomotor capacity is complex and that animals may show compromise phenotypes allowing them to deal with a variety of substrates in their natural environment. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **115**, 869–881.

ADDITIONAL KEYWORDS: acceleration – limb morphology – locomotion – speed – stamina.

INTRODUCTION

Locomotion is of paramount importance in animals because it has direct implications for fitness through its role in predator escape, prey capture, and territory defence (Miles, 2004; Husak, 2006; Husak & Fox, 2006; Montuelle *et al.*, 2012). Consequently, animal locomotion has been studied extensively in a wide variety of taxa (Irschick & Garland, 2001). Yet, despite its ecological relevance, locomotion has been studied most often under controlled laboratory conditions (Biewener, 2002). Although these studies are extremely insightful, the natural world is not homogenous and animals typically face varying obstacles,

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inclines, and differences in substrate properties. Moreover, animals may use different types of locomotion (e.g. jumping, acceleration, turning, etc.) in addition to the steady-state constant velocity locomotion most often studied on treadmills or race tracks (Biewener, 2002). As a result, more attention is currently being paid to locomotor behaviours such as manoeuvering, acceleration, jumping, and intermittent locomotion in general (Daley & Biewener, 2006; McGowan, Baudinette & Biewener, 2007; Higham, Korchari & McBrayer, 2011).

Over recent decades, significant attention has been paid to how variation in incline and substrate diameter affects locomotor capacity in a variety of vertebrate taxa (Huey & Hertz, 1984; Irschick & Jayne, 1998; Schmitt, 2003; Higham & Jayne, 2004; Spezzano & Jayne, 2004; Vanhooydonck, Herrel & Irschick, 2006; Foster & Higham, 2012; Hyams, Jayne & Cameron, 2012; Herrel *et al*., 2013). By contrast, how variation in substrate texture or roughness affects locomotion remains rather poorly understood (Vanhooydonck *et al*., 2005; Tulli, Abdala & Cruz, 2012; Cabezas-Cartes, Kubisch & Ibargüengoytía, 2014). Yet, natural substrates vary widely in texture and structure, and most animals have to deal with different substrates on a daily basis. Moreover, how different locomotor performance traits are affected by substrate properties remains virtually unstudied. The effects of substrate properties are most likely not the same for all types of locomotor performance (Vanhooydonck *et al*., 2006; but see Korff & McHenry, 2011). For example, granular substrates such as sand likely have a stronger impact on locomotor traits such as acceleration capacity than on steady-state velocity given that their physical properties change depending on the stress exerted on the substrate (Maladen *et al*., 2009; Mazouchova *et al*., 2010; Li, Hsieh & Goldman, 2012). As a result, many animals have evolved morphological and behavioural adaptations to be able to accelerate in or on such granular substrates (Luke, 1986; Maladen *et al*., 2009).

Because substrate properties affect locomotor capacity, animals are often adapted to the substrates that they move on most commonly. For example, *Anolis* lizards living on branches of different diameters show differences in limb length optimizing either speed or stability on their habitual substrate in the wild (Losos & Sinervo, 1989). Moreover, these adaptations have arisen independently on the different islands of the Greater Antilles, suggesting that the mechanical constraints imposed by substrate diameter drive the evolution of limb length in this group of lizards (Williams, 1983; Losos & Sinervo, 1989; Losos, 1990, 2009). Not only arboreal animals, but also desert dwelling animals and rock climbers typically show specialized morphologies allowing them to perform better on their natural substrates (Herrel, Meyers & Vanhooydonck, 2002; Goodman, Miles & Schwarzkopf, 2008; Revell *et al*., 2007). For example, many desert dwelling lizards have evolved toe fringes, providing them with an increased toe surface area. This has been suggested to provide them with better traction on fine granular substrates, although the results from experimental studies remain equivocal (Carothers, 1986; Luke, 1986; but see also Korff & McHenry, 2011).

In the present study, we explore how variation in substrate (sand, slate, cork) affects locomotor performance in lacertid lizards that differ in their body and limb shape (Fig. 1, Table 1). Lacertid lizards comprise an Old World radiation of lizards and have invaded a variety of microhabitats from sandy deserts to rocks, dense vegetation, and trees (Arnold, 1989). We also test whether substrate effects are the same for different types of locomotor performance (speed, acceleration, and endurance capacity). We selected our substrates to impose different constraints on locomotor performance. Sand is a granular substrate that will result in a lizard's feet slipping relative to the surface in nonspecialized species. Slate, on the other hand, is solid but extremely smooth and likely provides little friction. Cork was chosen as a substrate that provides excellent friction and allows interlocking of the claws with the substrate, which should induce optimal performance in all species. We predict, moreover, that the effects of substrate will be different for different locomotor traits. For example, deformable, granular substrates are predicted to result in a higher metabolic cost of locomotion because of an increase in mechanical work and a decrease in the efficiency of the positive work carried out by muscles and tendons (Lejeune, Willems & Heglund, 1998; Li *et al*., 2012). Given the higher metabolic cost, we predict that animals will fatigue sooner on a granular substrate such as sand and thus show a lower stamina. Similarly, granular substrates, as well as smooth substrates such as slate, providing little friction, should reduce acceleration capacity and speed. Indeed, maximal sprint speed and acceleration are likely affected strongly by the traction provided by the substrate and, as such, we predict that both locomotor performance traits will be lower on substrates providing little friction. However, note that, in contrast to acceleration, animals may be able to overcome the limitations of friction by taking shorter steps at higher frequency. Globally, we predict that (1) the different substrates will affect the different species to different degrees given their differences in morphology and (2) that different performance traits will be differentially affected by the different substrates.

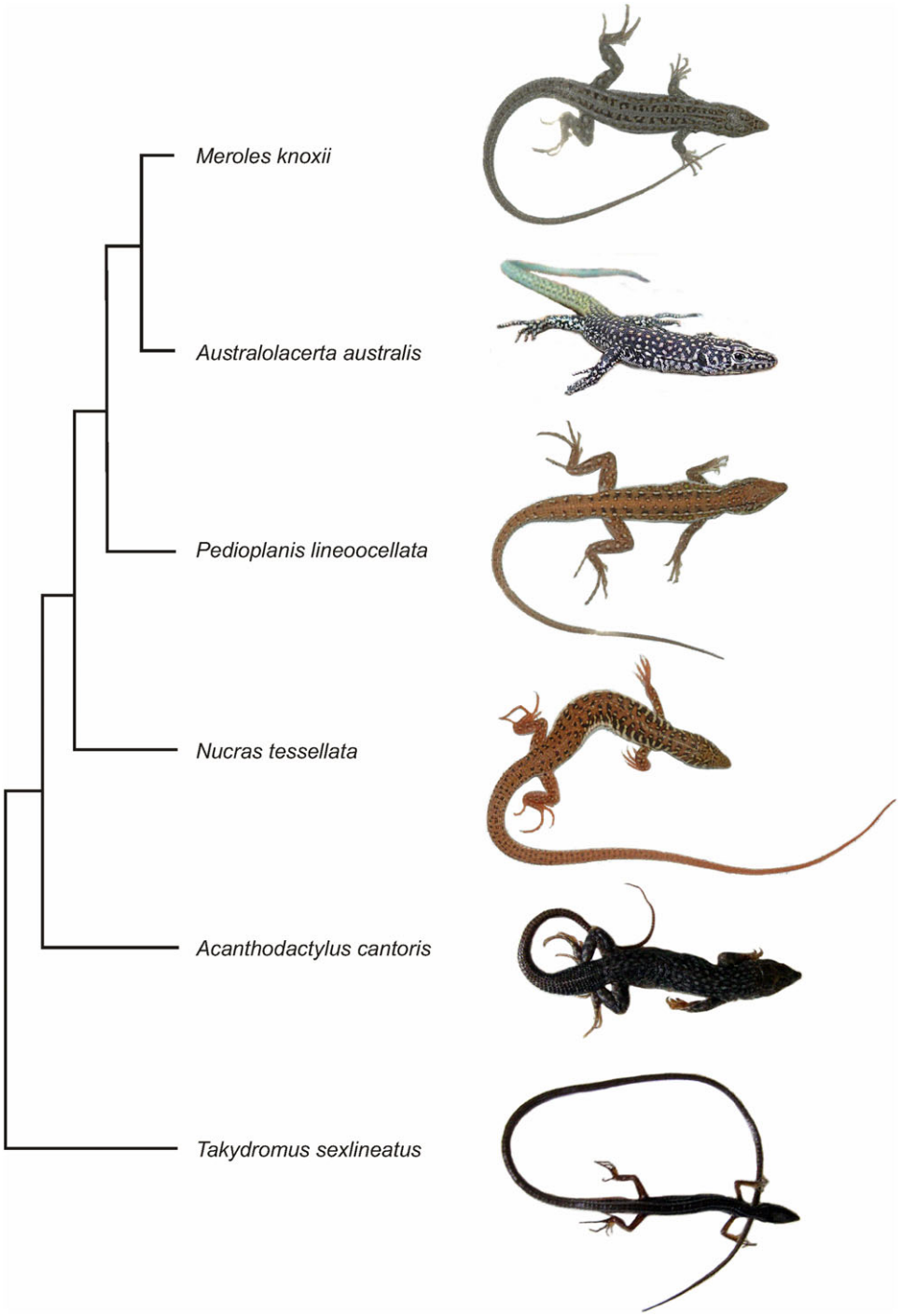

Figure 1. Phylogeny of the species included in the present study based on Kapli *et al*. (2011) and Edwards *et al*. (2012). To the right of the phylogeny are dorsal views of the different species illustrating the striking differences in body shape, limb length, and tail length across species. The *Australolacerta* australis image was kindly provided by A. Rebelo (Cape Town, South Africa).

MATERIAL AND METHODS

ANIMALS

Between November 2010 and June 2012, we quantified whole-organism locomotor performance in six lacertid species that were wild-caught (*Australolacerta australis* – Hewitt 1926, *Meroles knoxii* – Milne-Edwards 1829, *Nucras tessellata* – Smith 1838, and *Pedioplanis lineoocellata* – Duméril & Bibron

Table 1. Mean ± SE of snout–vent length (SVL), body mass, femur length, tibia length, metatarsus length, hind toe length, and total hind limb length per species (*N*) for individuals used in the locomotion trials

Species	SVL (mm)	Mass (g)	Femur (mm)	Tibia (mm)	Metatarsus (mm)	Toe (mm)	Hind limb (mm)	Habitat
Acanthodactylus cantoris (4)	53.64 ± 2.96	3.78 ± 0.70	11.58 ± 0.47	10.79 ± 0.39	3.92 ± 0.12	10.19 ± 0.46	36.48 ± 1.20	Sand
Australolacerta australis (9)	68.12 ± 1.58	7.21 ± 0.52	12.30 ± 0.39	10.02 ± 0.26	3.91 ± 0.10	9.89 ± 0.21	36.12 ± 0.86	Rocks
Meroles knoxii (10)	47.98 ± 0.64	2.33 ± 0.16	9.15 ± 0.16	8.66 ± 0.13	3.28 ± 0.08	7.94 ± 0.20	29.03 ± 0.47	Sand
Nucras tessellata (1)	61.29	4.63	10.86	9.96	3.83	10.27	34.92	Gravel
Pedioplanis lineoocellata (11)	50.07 ± 1.04	2.54 ± 0.19	10.80 ± 0.19	10.01 ± 0.17	3.84 ± 0.13	9.29 ± 0.22	33.94 ± 0.60	Gravel
Takydromus sexlineatus (10)	56.07 ± 0.90	3.50 ± 0.27	9.02 ± 0.15	8.40 ± 0.14	3.05 ± 0.056	8.77 ± 0.21	29.24 ± 0.43	Shrubs and grasses

1839) or obtained through the pet trade (*Acanthodactylus cantoris* – Günther 1864 and *Takydromus sexlineatus* – Daudin 1802) (Table 1). Although *A. cantoris* and *M. knoxii* are specialized sand dwellers from Near Asia and South-Africa, respectively (Anderson, 1963; Branch, 1998), *N. tessellata* and *P. lineoocellata* are typically found on gravel substrates in the South-African deserts (Branch, 1998). By contrast, *A. australis* is South-African endemic rock dweller commonly found on rocky outcroppings and rarely observed on the ground (Branch, 1998). Finally, *T. sexlineatus* is a species from South-East Asia that is typically found in dense grass and shrubs (Grismer, 2011). All lizards underwent trials on the same substrates in the same order.

We measured snout–vent length (SVL) from the tip of the snout to the cloaca, as well as femur length, tibia length, metatarsus length, and the length of the longest toe of each individual to the nearest 0.01 mm using digital calipers (Mitutoyo CD-15DC) (Vanhooydonck, Van Damme & Aerts, 2001). Total hind limb length was calculated as the sum of the different limb segments. Body mass was measured to the nearest gramme using a digital scale (Ohaus Scout Pro). We started the locomotor performance trials within 2 weeks after the animals arrived in the laboratory. Pet trade animals were purchased as soon as they arrived and transferred to the laboratory. As such, they did not spend a prolonged time in captivity before the onset of the trials. During experimentation, we did not observe any noticeable difference in performance capacity or behaviour between wild-caught or pet trade species. Lizards were kept in the animal room of the Laboratory of Functional Morphology at the University of Antwerp, under a 10 : 14 h dark/light cycle at 28 °C. Lizards were housed with a maximum of five individuals per cage in cages (0.5 × 1 m). Cages were equipped with a 75- or 100-W light bulb, according to the cage size, providing extra light and heat for 10 h per day. Rocks, branches, and leaf litter were scattered on the cage bottom to provide basking and hiding spots. Lizards were fed crickets (*Achetus domesticus*) dusted with calcium and vitamin supplements three times per week. In addition to providing water *ad libitum* in water bowls, cages were sprayed with water twice a week. Animals from South Africa were captured under permit numbers 0011-AAA004-00333 and 532-08-01-01/1–11-04. Experiments were carried out in accordance with procedures stipulated by the ethics committee of the University of Antwerp.

Sprint speed

We quantified sprint speed in accordance with standard procedures (Bauwens *et al.*, 1995; Vanhooydonck, Van Damme & Aerts, 2001). Each

individual was chased five times down a electronic racetrack (length 2 m, width 15 cm) equipped with photocells every 25 cm. The bottom of the racetrack was covered with cork, a layer of sand (depth 1 cm), and slates cut to the width of the race track, to provide substrates that differ in texture and roughness. Each individual thus ran 15 times across the track and only a single substrate was tested on a given day. All trials were performed between 10.00 h and 16.00 h when lizards were active. Each run was scored as 'good' or 'bad' depending on the individual's willingness to run (Van Berkum & Tsuji, 1987; Tsuji *et al*., 1989). We would call a run a 'good' run if the lizard ran continuously, in the middle of the track, over a distance of at least 50 cm; in a bad run, the animal frequently stopped and turned, and/or jumped from side to side. Bad runs were not included in further analyses, resulting in the exclusion of one *A. australis*, one *M. knoxii*, three *P. lineoocellata*, and one *T. sexlineatus* from the results. This resulted in data for four *A. cantoris*, eight *A. australis*, nine *M. knoxii*, one *N. tessellata*, eight *P. lineoocellata*, and nine *T. sexlineatus*. Prior to the start of the experiment, and between trials, lizards were placed in an incubator set at 35 °C for at least 1 h. These temperatures are within the range of field-active body temperatures (Castilla, Van Damme & Bauwens, 1999) and within the optimal performance range of the different species. As an estimate of a lizard's maximal sprinting capacity, we used maximal speed attained over any 50-cm interval of the five trials.

Acceleration

We quantified the acceleration capacity of each lizard on a 2-m long racetrack with a cork, sand, and slate substrate. A dot was painted dorsally, at the level of the hip on each individual lizard, using a nontoxic white paint pen, to facilitate subsequent digitization. Each individual was tested five times on each of the three substrates. Prior to the start of the experiment and between trials, lizards were placed in an incubator set at 35 °C for at least 1 h. A high-speed video camera (Redlake Motionscope; 1280 × 1024 pixel resolution; IDT Inc.) set at 500 Hz was placed 1 m above the track so that the first 0.5 m of the track was in view, filming the lizards dorsally when accelerating from a standstill. A scaling grid of known dimensions was filmed before the start of each filming session and was digitized (PROANALYST, version 1.5.3.8; Xcitex) to calculate the scaling factor. Clips were subsequently digitized at 500 Hz, using the (semi-)automatic tracking option in PROANALYST. Digitization started at least 20 frames prior to any movement by the lizard and stopped when the lizard ran out of view (Fig. 2). We subsequently exported the scaled (m) displacement coordinates to EXCEL (Microsoft Corp.) and filtered the data using a fourth-order zero-phase shift Butterworth low-pass data noise filter (VBA for EXCEL; Van Wassenbergh, 2007) at 40 Hz. Based on the filtered data, the first and second derivatives of displacement against time were calculated and the latter was used as a measure of instantaneous acceleration (Fig. 2). In 99% of the cases, the acceleration profile (i.e. instantaneous acceleration plotted over time) showed a clear peak before the end of the sequence. Cases in which this was not true were not withheld for further analyses. As an estimate of an individual's maximum acceleration capacity, we used the maximum instantaneous acceleration out of any of the five trials. This resulted in data for four *A. cantoris*, ten *A. australis*, ten *M. knoxii*, one *N. tessellata*, five *P. lineoocellata*, and nine *T. sexlineatus*.

Stamina

We quantified stamina on a circular track with a cork and sand substrate and a circumference of 2.5 m (Huyghe *et al*., 2007; Herrel & Bonneaud, 2012). Because it was impossible to construct a circular track with slate substrate, animals were tested on only two substrates. Each individual was tested three times with trials spread over two consecutive days. We induced lizards to run on the circular track at a voluntary, self-chosen speed that was clearly submaximal in all species. A trial was considered terminated if a lizard did not respond to multiple taps on the tail base and did not show a righting response when placed on its back (Huey *et al*., 1990). Prior to the start of the experiment, lizards were placed in an incubator set at 35 °C for at least 1 h. Between trials on the same day, this period was extended to at least 2 h. As an estimate of a lizard's maximal stamina, we used the maximal distance run to exhaustion (m) out of the three trials. This resulted in data for four *A. cantoris*, ten *A. australis*, ten *M. knoxii*, one *N. tessellata*, seven *P. lineoocellata*, and eight *T. sexlineatus*.

Statistical analysis

All data were $\log_{10}$-transformed before analysis. All analyses were performed using SPSS, version 20 (IBM Corp.). First, we tested whether species differed in body size (SVL and mass) using univariate analysis of variance (ANOVA). Next, we tested whether species differed in limb morphology using a multivariate analysis of covariance (MANCOVA) with SVL as co-variate. These analyses were run both with and without *N. tessellata*. Given that we only had a single *N. tessellata*, we next removed this

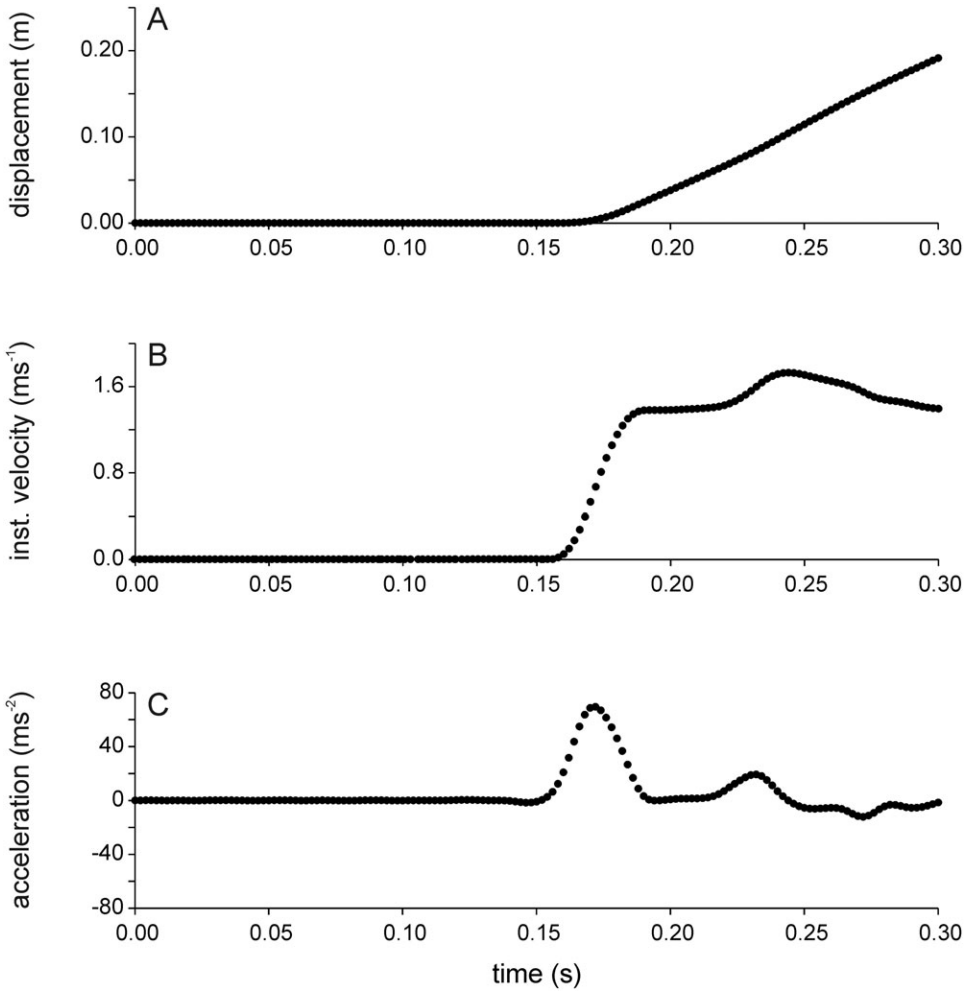

Figure 2. Raw displacement (A), instantaneous velocity (B), and acceleration (C) plots for a single acceleration sequence in *Australolacerta australis* used to calculate peak accelerations.

species from the analyses and ran regression analyses with SVL as independent variable and mass and limb dimensions as dependents and saved unstandardized residuals. Finally, we ran a multivariate analysis of variance (MANOVA) on the residuals to test for differences between species coupled to univariate ANOVAs and Bonferroni post-hoc tests to explore which species differed from one another.

To test for differences in performance on the different substrates, we performed repeated-measures ANOVAs to test whether substrate differently affected speed, acceleration, and stamina in the six lacertid species. Maximum speed, acceleration, and stamina per individual were entered as dependent variables, the different substrate types as within-subject variables, and species as between-subject variables. Given that we only had a single *N. tessellata*, we ran all analyses with and without this species to make sure that its presence did not bias the results.

RESULTS

Morphometrics

Species differed in SVL ($F_{5,39} = 32.68$; $P < 0.001$) and body mass ($F_{5,39} = 24.20$; $P < 0.001$). Inspection of the means showed that *A. australis* was the biggest and heaviest species and *P. lineoocellata* and *M. knoxii* the smallest species in our analyses. A MANCOVA detected significant differences in limb dimensions (Wilks' lambda = 0.10, $F_{25,128} = 4.30$; $P < 0.001$). Univariate ANOVAs showed that species differed in all limb dimensions. A MANOVA on the residual data excluding *N. tessellata* also showed significant differences between species (Wilks' lambda = 0.11, $F_{24,120} = 4.36$; $P < 0.001$). Univariate ANOVAs subsequently showed that species differed in all traits except residual body mass. Post-hoc tests indicated that *T. sexlineatus* had a significantly shorter relative femur and metatarsus than all other species. Additionally, *M knoxii* was also significantly

different from all other species and had the second shortest relative femur and metatarsus lengths. However *A. australis*, *A. cantoris*, and *P. lineoocellata* did not differ from one another in relative femur length. Similarly, *T. sexlineatus* and *M. knoxii* had a relatively short tibia. Although *T. sexlineatus* was different from all others except *M. knoxii*, *M. knoxii* differed from *A. cantoris* and *P. lineoocellata* only. The longest relative tibia was observed in *A. cantoris*, which differed from all species except *P. lineoocellata*. The longest toe was also relatively shorter in *T. sexlineatus* and *M. knoxii* compared to all other species. Although *A. australis* was not different from any of the other species, *A. cantoris* and *P. lineoocellata* had significantly longer toes than the two species with the shortest toes. Overall, this resulted in *T. sexlineatus* and *M. knoxii* having the shortest relative hind limb lengths and being significantly different from *A. australis*, *A. cantoris*, and *P. lineoocellata*, which had the longest relative hind limb lengths.

Performance

The mean ± SE for each performance trait, on each of the three substrates, is given per species in Table 2. An analysis of maximal acceleration capacity on the different substrates detected a significant effect of substrate on acceleration capacity (Wilks' lambda = 0.72; $F_{2,32} = 6.17$; $P = 0.005$; without *N. tessellata*: Wilks' lambda = 0.65; $F_{2,31} = 8.48$; $P = 0.001$). No interaction between substrate and species was observed (Wilks' lambda = 0.90; $F_{8,64} = 0.43$; $P = 0.90$). Indeed, all species showed a decrease in acceleration capacity on sand compared to the two other substrates (Fig. 3). The species effect was, however, significant ($F_{5,34} = 8.55$; $P < 0.001$; without *N. tessellata*: $F_{4,33} = 10.66$; $P < 0.001$). Thus, acceleration capacity differs among species. Speed over 50 cm showed a significant substrate by species interaction effects (Wilks' lambda = 0.47; $F_{10,64} = 2.95$; $P = 0.04$; without *N. tessellata*: Wilks' lambda = 0.47; $F_{8,64} = 3.64$; $P = 0.002$) (Fig. 4), suggesting that the effect of substrate was not the same for all species, with some running faster and others slower on some of the substrates. Substrate effects were also significant (Wilks' lambda = 0.75; $F_{2,32} = 5.37$; $P = 0.01$; without *N. tessellata*: Wilks' lambda = 0.59; $F_{2,32} = 11.00$; $P < 0.001$), indicating that the substrate influenced the maximal speed over 50 cm that a lizard could attain. Species differed significantly in the speed over 50 cm ($F_{5,34} = 13.43$; $P < 0.001$; without *N. tessellata*: $F_{4,33} = 15.44$; $P < 0.001$). Stamina measured as the distance run until fatigue showed a significant interaction effect (Wilks' lambda = 0.68; $F_{5,34} = 3.26$; $P = 0.016$; without *N. tessellata*: Wilks' lambda = 0.67; $F_{4,34} = 4.05$; $P = 0.009$), with some species running for a longer time on cork, yet others running longer on sand (*M. knoxii* and *T. sexlineatus*) (Fig. 5).

DISCUSSION

Our results indicate that substrate effects are species-dependent for the maximal speed over 50 cm and the distance run to exhaustion as indicated by the significant interaction effects. However, this was not the case for acceleration capacity, suggesting that substrate texture differentially affects burst performance (acceleration) vs. more long duration measures of locomotor performance (distance run to exhaustion, maximal speed over 50 cm). We initially predicted that endurance capacity should be lower on granular substrates as a result of the potential higher metabolic cost of locomotion associated with running on granular substrates (Lejeune *et al.*, 1998), although this was not the case for all species. Similarly, we predicted that sand should reduce acceleration capacity, which is what was observed in all species.

Acceleration capacity was most strongly affected by substrate structure and uniformly so: for most species, acceleration capacity was highest on the cork substrate, lowest on the sand, and intermediate on slate. Only *N. tessellata* showed a higher acceleration capacity on slate vs. the other substrates tested, which may be a result of its unusual elongated body shape (Bergmann & Irschick, 2010), allowing it to use C- or S-like fast starts where bends in the body help propel the animal forward. However, given that we measured only a single individual, these results should be interpreted with caution. Although comparative data remain scarce, a previous study on locomotion in geckos showed similar effects of the substrate on acceleration capacity (Vanhooydonck *et al.*, 2005). Although, in uphill running geckoes, the effects of substrate texture were strong for acceleration and instantaneous speed, final speed (comparable to our measures of sprint speed) showed no substrate effects. This suggests that substrate texture and the amount of friction that a lizard can generate may be of greater importance during locomotor behaviours where force exchanges with the substrate are large, such as during climbing or accelerating from a standstill (Mazouchova *et al.*, 2010).

For maximal speed over 50 cm, a significant interaction effect was observed. Although *A. cantoris*, *T. sexlineatus*, and *P. lineoocellata* increased their maximal speed from cork over sand to slate, *M. knoxii* had the highest speed on cork and lower

Table 2. Summary of the performance data on different substrates

Performance	Substrate	Species (*N*)					
		Acanthodactylus cantoris (4)	*Australolacerta australis* (10)	*Meroles knoxii* (10)	*Nucras tessellata* (1)	*Pedioplanis lineoocellata* (5)	*Takydromus sexlineatus* (9)
Acceleration (m s^{-2})	Cork	72.48 ± 6.76	51.83 ± 4.76	40.44 ± 2.31	35.19	36.20 ± 3.33	38.55 ± 6.12
	Sand	48.67 ± 8.92	34.95 ± 3.14	35.22 ± 3.58	23.07	33.42 ± 6.76	24.13 ± 2.68
	Slate	62.75 ± 7.44	43.43 ± 4.74	37.91 ± 2.72	52.61	33.62 ± 5.05	31.95 ± 3.06
		Acanthodactylus cantoris (4)	*Australolacerta australis* (8)	*Meroles knoxii* (9)	*Nucras tessellata* (1)	*Pedioplanis lineoocellata* (8)	*Takydromus sexlineatus* (9)
Sprint $speed_{50}$ (m s^{-1})	Cork	1.97 ± 0.24	0.86 ± 0.08	1.89 ± 0.19	2.50	0.91 ± 0.14	0.58 ± 0.12
	Sand	2.27 ± 0.12	1.67 ± 0.09	1.57 ± 0.19	2.08	1.07 ± 0.31	0.68 ± 0.13
	Slate	2.62 ± 0.15	1.53 ± 0.06	1.51 ± 0.14	2.63	1.32 ± 0.10	0.97 ± 0.16
		Acanthodactylus cantoris (4)	*Australolacerta australis* (10)	*Meroles knoxii* (10)	*Nucras tessellata* (1)	*Pedioplanis lineoocellata* (7)	*Takydromus sexlineatus* (8)
Stamina (m)	Cork	31.38 ± 3.47	32.40 ± 3.49	12.05 ± 1.96	31.00	13.68 ± 3.76	15.47 ± 1.58
	Sand	27.31 ± 3.49	22.05 ± 1.71	16.45 ± 2.22	24.50	10.82 ± 3.17	17.75 ± 2.73

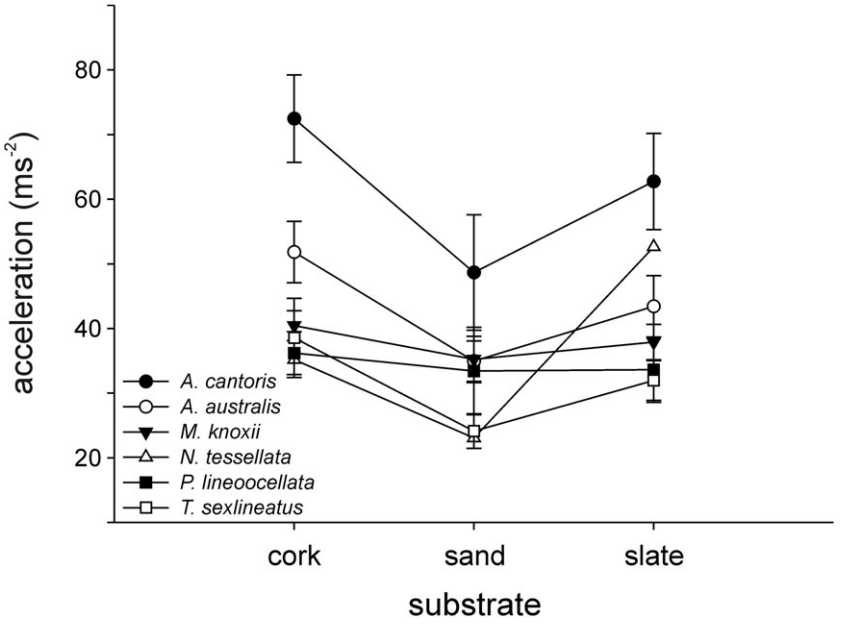

Figure 3. Scatter plot illustrating the peak acceleration capacity of the different species on three different substrates (cork, sand, and slate). Note how the acceleration on sand is lower for most species except *Pedioplanis and Meroles*, which perform equally well on sand and on slate.

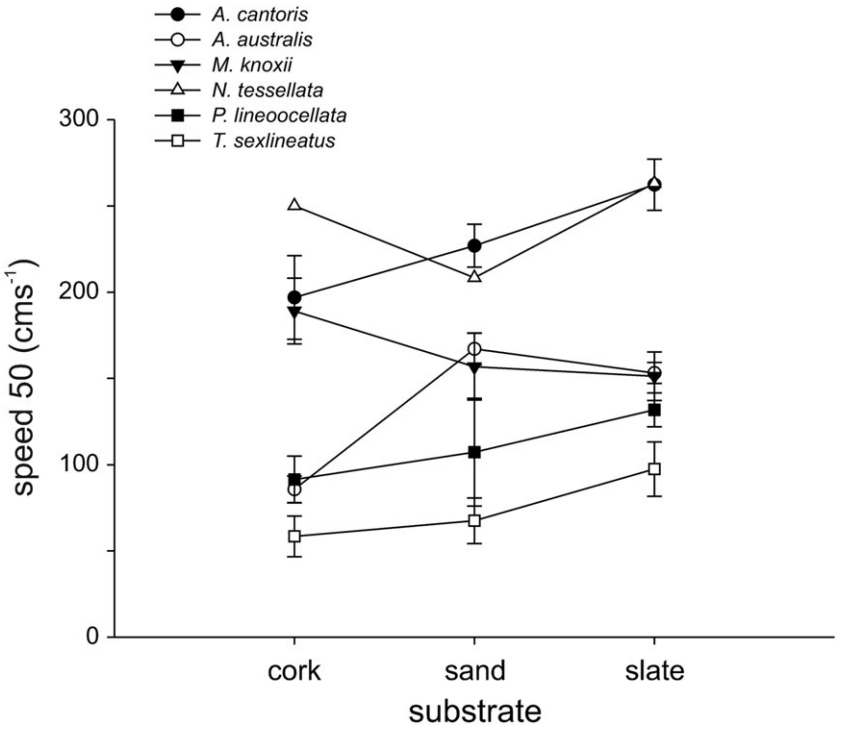

Figure 4. Scatter plot illustrating sprint speed capacity on different three different substrates (cork, sand, and slate). Speed over 50 cm shows significant interaction effects between substrate and species. For example, whereas *Nucras tessellata* shows its lowest performance on sand, *Australolacerta australis* performs best on this substrate.

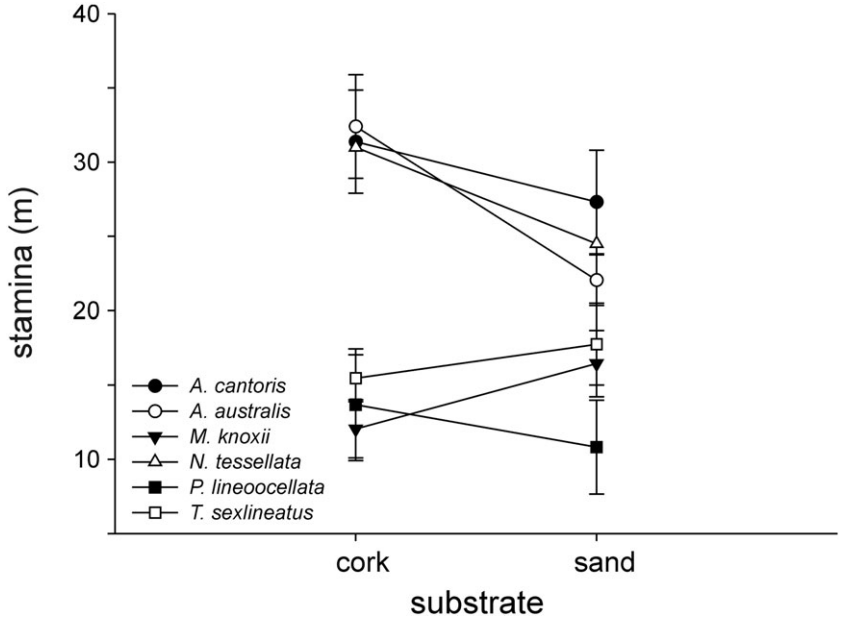

Figure 5. Scatter plot illustrating differences in stamina of lizards running on two different substrates (cork and sand). Although *Australolacerta, Nucras, Acanthodactylus,* and *Pedioplanis* show a decreased stamina on sand compared to cork, *Meroles* and *Takydromus* show a slight increase in stamina on sand vs. on cork.

speeds on sand and slate. Moreover, *A. australis* showed its lowest speed on cork in contrast to *N. tessellata*, which showed its lowest speed on sand. Thus, most animals, with the exception of *M. knoxii*, were capable of attaining relatively high speeds over 50 cm on smooth substrates such as slate (Fig. 4). This is in accordance with studies on the effect of substrate roughness on speed in other small legged animals (i.e. hatchling turtles and lizards; Kohlsdorf *et al*., 2004; Renous *et al*., 2010; Bergmann & Irschick, 2010; Mazouchova *et al*., 2010; Korff & McHenry, 2011; Tulli *et al*., 2012) and robots (Li, Zhang & Goldman, 2013). By contrast, locomotor performance appears to be detrimentally affected by granular media in larger, heavier organisms (humans: Lejeune *et al*., 1998; adult turtles: Claussen *et al*., 2002; 2.3-kg robots: Li *et al*., 2009, 2013) or during acceleration (present study).

Our results indicate that interaction effects are also significant for stamina. Thus, whether stamina is higher on a granular substrate vs. cork is dependent on the species. Although *T. sexlineatus* and *M. knoxii* show better endurance on sand compared to other substrates, the other species consistently performed best on a cork substrate (Fig. 4). Unexpectedly, the differential effect of a sandy substrate on stamina does not reflect the species' microhabitat use because *T. sexlineatus* is a grass-dweller. Although *M. knoxii* is indeed a sand-dwelling species, other desert species such as *A. cantoris* showed a reduced performance on sand compared to other substrates despite their specialized morphology including the presence of toe fringes. Thus, rather than being specialized, it may be that it is simply a result of these two species (*T. sexlineatus* and *M. knoxii*) having the lowest body weight and shortest limbs of the species included in the present study (Table 1). A lower body weight and shorter limbs likely also imply lower locomotor forces. Thus, for these animals, the substrate may not behave as a granular one that generates slip but, in contrast, provides excellent friction (Li *et al*., 2013).

In general, it appears that habitat specialization does not play a role in determining how a species will perform on the different substrates used in the present study. It should be noted, however, that our sample size is small and, as such, other species may show different patterns, which needs to be tested. For example, most species had higher speeds over 50 cm on the slate compared to the other substrates except the only rock-dweller in the present study (*A. australis*), which performed best on sand. The sand-dweller, *M. knoxii*, on the other hand, performed best on a cork substrate rather than on sand. Similarly, acceleration capacity was not maximal on a sandy substrate for the two species that live in sandy habitats (*A. cantoris*, *M. knoxii*) and all species showed a reduced acceleration performance on sand compared to the other substrates. This mimics results for a study on South-American lizards where

it was noted that 'surprisingly, no ecological group performed better on the surface resembling its own habitat' (Tulli *et al*., 2012). Taken together, this suggests that performance is principally driven by the dynamics of locomotion (i.e. locomotor forces, body weight, substrate yield forces) rather than specializations for habitat use. Animals may then simply try to minimize any negative effects of the substrate they move on habitually through morphological or behavioural specializations or adjustments of locomotor kinematics (Bergmann & Irschick, 2010; Mazouchova *et al*., 2010). Thus, the habitats and substrates used in sand-dwelling lizards may be decoupled from locomotor capacity as suggested previously (Korff & McHenry, 2011). Morphological specializations such as the presence of toe fringes, however, may allow a specialist sand-dweller such as *A. cantoris* to have a relatively high acceleration capacity compared to other species (lacking toe fringes) on sand despite its large body mass (Fig. 3).

In summary, substrate effects on locomotor performance were ubiquitous but differed depending on the type of locomotor behaviour examined. Although the burst performance trait acceleration capacity resulted in a 'global' effect that is generally similar for all species, steady-state types of performance resulted in more complex patterns that depend on the morphology, behaviour, and kinematics of locomotion of the different species.

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REFERENCES

Anderson SC. 1963. Amphibians and reptiles from Iran. *Proceedings of the California Academy of Sciences Series* **4:** 417–498.

Arnold EN. 1989. Towards a phylogeny and biogeography of the Lacertidae: relationships within an Old-World family of lizards derived from morphology. *Bulletin of the British Museum Natural History* **55:** 209–257.

Bauwens D, Garland TJ, Castilla AM, Van Damme R. 1995. Evolution of sprint speed in lacertid lizards: morphological, physiological and behavioral covariation. *Evolution* **49:** 848–863.

Bergmann PJ, Irschick DJ. 2010. Alternate pathways of body shape evolution translate into common patterns of locomotor evolution in two clades of lizards. *Evolution* **64:** 1569–1582.

Biewener AA. 2002. Future directions for the analysis of musculoskeletal design and locomotor performance. *Journal of Morphology* **252:** 38–51.

Branch B. 1998. *Field guide to the snakes and other reptiles of Southern Africa*. Fort Myers, FL: Ralph Curtis Books Publishing.

Cabezas-Cartes F, Kubisch EL, Ibargüengoytía NR. 2014. Consequences of volcanic ash deposition on the locomotor performance of the *Phymaturus spectabilis* lizard from Patagonia Argentina. *Journal of Experimental Zoology* **321A:** 164–172.

Carothers JH. 1986. An experimental confirmation of morphological adaptation: toe fringes in the sand dwelling lizard *Uma scoparia*. *Evolution* **40:** 871–874.

Castilla AM, Van Damme R, Bauwens D. 1999. Field body temperatures, mechanisms of thermoregulation and evolution of thermal characteristics in lacertid lizards. *Natura Croatica* **8:** 253–274.

Claussen DL, Lim R, Kurz M, Wren K. 2002. Effects of slope, substrate, and temperature on the locomotion of the ornate box turtle, *Terrapene ornata*. *Copeia* **2002:** 411–418.

Daley MA, Biewener AA. 2006. Running over rough terrain reveals limb control for intrinsic stability. *Proceedings of the National Academy of Sciences* **103:** 15681–15686.

Edwards S, Vanhooydonck B, Herrel A, Measey GJ, Tolley KA. 2012. Convergent evolution associated with habitat decouples phenotype from phylogeny in a clade of lizards. *Plos ONE* **7:** e51636.

Foster KL, Higham TE. 2012. How forelimb and hindlimb function changes with incline and perch diameter in the green anole, *Anolis carolinensis*. *Journal of Experimental Biology* **215:** 2288–2300.

Goodman BA, Miles DB, Schwarzkopf L. 2008. Life on the rocks: habitat use drives morphological and performance evolution in lizards. *Ecology* **89:** 3462–3471.

Grismer LL. 2011. *Lizards of Peninsular Malaysia, Singapore and their adjacent archipelagos*. Frankfurt: Edition Chimaira.

Herrel A, Bonneaud C. 2012. Trade-offs between burst performance and maximal exertion capacity in a wild amphibian, *Xenopus tropicalis*. *Journal of Experimental Biology* **215:** 3106–3111.

Herrel A, Meyers JJ, Vanhooydonck B. 2002. Relations between micro habitat use and limb shape in phrynosomatid lizards. *Biological Journal of the Linnean Society* **77:** 149–163.

Herrel A, Perrenoud M, Decamps T, Abdala V, Manzano A, Pouydebat E. 2013. The effect of substrate diameter and incline on locomotion in an arboreal frog. *Journal of Experimental Biology* **216:** 3599–3605.

Higham TE, Jayne BC. 2004. Locomotion of lizards on inclines and perches: hind limb kinematics of an arboreal specialist and a terrestrial generalist. *Journal of Experimental Biology* **207:** 233–248.

Higham TE, Korchari PG, McBrayer LD. 2011. How to climb a tree: lizards accelerate faster, but pause more, when escaping on vertical surfaces. *Biological Journal of the Linnean Society* **102:** 83–90.

Huey RB, Dunham AE, Overall KL, Newman RA. 1990. Variation in locomotor performance of demographically known populations of the lizard *Sceloporus merriami*. *Physiological Zoology* **63:** 845–872.

Huey RB, Hertz PE. 1984. Effects of body size and slope on acceleration of a lizard (*Stellio* (*Agama*) *stellio*). *Journal of Experimental Biology* **110:** 113–123.

Husak JF. 2006. Does speed help you survive? A test with collared lizards of different ages. *Functional Ecology* **20:** 174–179.

Husak JF, Fox SF. 2006. Field use of maximal sprint speed by collared lizards (*Crotaphytus collaris*): compensation and sexual selection. *Evolution* **60:** 1888–1895.

Huyghe K, Vanhooydonck B, Herrel A, Tadic Z, Van Damme R. 2007. Morphology, performance, behavior and ecology of three color morphs in males of the lizard *Podarcis melisellensis*. *Integrative and Comparative Biology* **47:** 211–220.

Hyams SE, Jayne BC, Cameron GN. 2012. Arboreal habitat structure affects locomotor speed and path choice of white-footed mice (*Peromyscus leucopus*). *Journal of Experimental Zoology A* **317:** 540–551.

Irschick DJ, Jayne BC. 1998. Effects of incline on acceleration, body posture, and hind limb kinematics in two species of lizard, *Callisaurus draconoides* and *Uma scoparia*. *Journal of Experimental Biology* **201:** 273–287.

Irschick DJ, Garland TJ. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Reviews of Ecology and Systematics* **32:** 367–396.

Kapli P, Poulakakis N, Lymberakis P, Mylonas M. 2011. A re-analysis of the molecular phylogeny of Lacertidae with currently available data. *Basic and Applied Herpetology* **25:** 97–104.

Kohlsdorf T, James RS, Carvalho JE, Wilson RS, Silva MDP, Navas CA. 2004. Locomotor performance of closely related *Tropidurus* species: relationships with physiological parameters and ecological divergence. *Journal of Experimental Biology* **207:** 1183–1192.

Korff WL, McHenry MJ. 2011. Environmental differences in substrate mechanics do not affect sprinting performance in sand lizards (*Uma scoparia* and *Callisaurus draconoides*). *Journal of Experimental Biology* **214:** 122–130.

Lejeune TM, Willems PA, Heglund NC. 1998. Mechanics and energetics of human locomotion on sand. *Journal of Experimental Biology* **201:** 2071–2080.

Li C, Hsieh T, Goldman DI. 2012. Multifunctional foot use during running in the zebra-tailed lizard (*Callisaurus draconoides*). *Journal of Experimental Biology* **215:** 3293–3308.

Li C, Umbanhowar PB, Komsuoglu H, Koditschek DE, Goldman DI. 2009. Sensitive dependence of the motion of a legged robot on granular media. *Proceedings of the National Academy of Sciences* **106:** 3029–3034.

Li C, Zhang T, Goldman DI. 2013. A terradynamics of legged locomotion on granular media. *Science* **339:** 1408–1412.

Losos JB. 1990. The evolution of form and function: morphology and locomotor performance ability in West Indian *Anolis* lizards. *Evolution* **44:** 1189–1203.

Losos JB. 2009. *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. Berkeley, CA: University of California Press.

Losos JB, Sinervo B. 1989. The effects of morphology and perch diameter on sprint performance of *Anolis* lizards. *Journal of Experimental Biology* **145:** 23–30.

Luke C. 1986. Convergent evolution of lizard toe fringes. *Biological Journal of the Linnean Society* **27:** 1–16.

Maladen RD, Ding Y, Li C, Goldman DI. 2009. Undulatory swimming in sand: subsurface locomotion of the sandfish lizard. *Science* **325:** 314–318.

Mazouchova N, Gravish N, Savu A, Goldman DI. 2010. Utilization of granular solidification during terrestrial locomotion of hatchling sea turtles. *Biology Letters* **6:** 398–401.

McGowan CP, Baudinette RV, Biewener AA. 2007. Modulation of proximal muscle function during level versus incline hopping in tammar wallabies (*Macropus eugenii*). *Journal of Experimental Biology* **210:** 1255–1265.

Miles DB. 2004. The race goes to the swift: fitness consequences of variation in sprint performance in juvenile lizards. *Evolutionary Ecology Research* **6:** 63–75.

Montuelle S, Herrel A, Libourel P-A, Daillie S, Bels VL. 2012. Flexibility in locomotor-feeding integration during prey capture in varanid lizards: effects of prey size and velocity. *Journal of Experimental Biology* **215:** 3823–3835.

Renous S, Hofling E, Da Rocha PLB. 2010. Effect of the substrate on the locomotion behavior of the South American iguanian lizard *Polychrus acutirostris*. *Italian Journal of Zoology* **77:** 216–226.

Revell LJ, Johnson MA, Schulte JA II, Kolbe JJ, Losos JB. 2007. A phylogenetic test for adaptive convergence in rock-dwelling lizards. *Evolution* **61:** 2898–2912.

Schmitt D. 2003. Substrate size and primate forelimb mechanics: implications for understanding the evolution of primate locomotion. *International Journal of Primatology* **24:** 1023–1036.

Spezzano LC, Jayne BC. 2004. The effects of surface diameter and incline on the hind limb kinematics of an arboreal lizard (*Anolis sagrei*). *Journal of Experimental Biology* **207:** 2115–2131.

Tsuji JS, Huey RB, van Berkum FH, Garland TJ, Shaw RG. 1989. Locomotor performance of hatchling fence lizards (*Sceloporus occidentalis*): quantitative genetics and morphometric correlates. *Evolutionary Ecology* **3:** 240–252.

Tulli MJ, Abdala V, Cruz FB. 2012. Effects of different substrates on the sprint performance of lizards. *Journal of Experimental Biology* **215:** 774–784.

Van Berkum FH, Tsuji JS. 1987. Inter-familiar differences in sprint speed of hatchling *Sceloporus occidentalis* (Reptilia: Iguanidae). *Journal of Zoology* **212:** 511–519.

Van Wassenbergh S. 2007. *VBA Excel add*. Available at: https://www.uantwerp.be/en/staff/sam-vanwassenbergh/my-website/excel-vba-tools

Vanhooydonck B, Andronescu A, Herrel A, Irschick DJ. 2005. Effects of substrate structure on speed and acceleration capacity in climbing geckos. *Biological Journal of the Linnean Society* **85:** 385–393.

Vanhooydonck B, Herrel A, Irschick DJ. 2006. Out on a limb: the differential effect of substrate diameter on acceleration capacity in *Anolis* lizards. *Journal of Experimental Biology* **209:** 4515–4523.

Vanhooydonck B, Van Damme R, Aerts P. 2001. Speed and stamina trade-off in lacertid lizards. *Evolution* **55:** 1040–1048.

Williams EE. 1983. Ecomorphs, faunas, island size, and diverse end points in island radiations of *Anolis*. In: Huey RB, Pianka ER, Schoener TW, eds. *Lizard ecology: studies of a model organism*. Cambridge, MA: Harvard University Press, 326–370.